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FINAL TECHNICAL REPORT

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GRANT TITLE: Ocean Environmental and Surface Parameters Controlling Initial Events in Macrofouler Recruitment

PERIOD OF PERFORMANCE: 15 Jaunary 1988 - 31 January 1991

OBJECTIVE: The principal long term objective of this project was to identify and characterize those environmental, biological, and surface parameters that control the initial and rate-limiting processes of larval settlement, attachment, metamorphosis, growth and recruitment of macrofouling organisms on surfaces immersed in the ocean.

ACCOMPLISHMENTS: Macrofouling of surfaces immersed in the ocean is initiated by the delivery and attachment of larvae or propagules from the plankton. Work in our laboratory conducted with support from an earlier contract from ONR revealed that settlement, attachment and metamorphosis of larvae of the mollusc, *Haliotis rufescens*, are controlled by the interaction between two chemosensory pathways. Receptors of the morphogenetic pathway recognize a surface-associated biochemical morphogen, produced by certain marine algae and bacteria, that is required to induce larval settlement, attachment and metamorphosis. The morphogenetic pathway receptors have low affinity, and are present in large number, suggesting a fail-safe mechanism for recognition of inductive surfaces. When high-affinity receptors of the amplifier pathway detect specific amino acids dissolved in low concentration in the seawater, the sensitivity of the larvae to low concentrations of the required surface-associated morphogen is amplified ca. 100-fold. *In vitro* resolution of the receptors and signal transducers of these two pathways confirm the results obtained *in vivo*.

Our recent studies in the ocean environment and the laboratory, conducted under grant N00014-88-K-0288, revealed that complex interactions between chemical and physical factors regulate larval settlement, attachment, metamorphosis and recruitment in the gregarious tube-building polychaete, *Phragmatopoma californica*. This organism is a significant macrofouler, capable of rapidly forming massive cemented aggregations on a variety of immersed surfaces. Larval settlement, attachment and metamorphosis are preferentially induced by contact-dependent chemical recognition of the freshly cemented tubes produced by the conspecific animals (thus explaining the "explosive" gregarious recruitment). Using an innovative procedure for significant purification of the natural morphogenic inducer, we found that the inducer is associated with the cement, and little or no other organic material. The cement was found to be a polyphenolic adhesive protein, with a high content of DOPA residues that are enzymatically cross-linked to form the final adhesive. In collaboration with Prof. Herbert Waite (U. DE), the amino acid sequences of the repeating DOPA-containing peptides of the adhesive protein have been determined. We found that DBMP, an aromatic compound known to form oxidation intermediates and cross-links closely analogous to those implicated in the enzyme-catalyzed oxidative cross-linking of DOPA residues within adhesive proteins, is a potent inducer of settlement, attachment, and metamorphosis of *P. californica* larvae in the ocean as well as in the laboratory. This is the first instance in which a defined organic chemical, identified in the laboratory as an inducer (or precursor to an inducer) of larval settlement and metamorphosis has been shown to exhibit these activities in the ocean. The results further demonstrate that larvae of this macrofouler do use chemical recognition of substrata to control attachment and metamorphosis in the natural ocean environment.

We have found that compounds that elevate intracellular cyclic AMP in many cell types also induce settlement and metamorphosis of *P. californica*, as does the direct ionic depolarization of externally excitable larval cells. These results suggest that the chemical signal of morphogen recognition may be transduced by changes in intracellular cyclic AMP and subsequent ionic depolarization, leading to the excitatory firing of primary chemosensory nerve cells; a similar mechanism of signal transduction has been implicated in the morphogenetic pathway in *Haliotis* larvae, and in several other species as well. Recently, other workers have suggested that certain free fatty acids, which they extracted and concentrated from biologically contaminated sources, also may be involved in the induction of larval settlement and metamorphosis in *P. californica*. However, these compounds are known to elevate cyclic AMP by non-specific effects on cell membranes in a wide variety of systems. In view of our evidence suggesting that cyclic AMP may be involved in the control of metamorphosis of *P. californica* larvae, it is possible that concentrated fatty acids may exert their effect on the larvae simply by membrane perturbation elevating cyclic AMP. In addition, we have found that fatty acids are not normally present in association with the natural inducer of *Phragmatopoma* settlement; when introduced by contamination, they induce settlement non-specifically.

Oceanic and environmental factors influencing the planktonic delivery of larvae have been investigated, and the principal controlling factors have been identified. We discovered that "Supply-Side" delivery accounts for only a small fraction of recruitment variability. Temporal variations in recruitment of *P. californica* are only partially (ca. 11%, $P = 0.2$) explained by temporal variations in larval delivery. Of the many chemical and physical oceanographic parameters investigated, variations in amino acid concentrations in the DOM, in conjunction with variations in larval density, were found to most accurately predict temporal variations in recruitment. Concentrations of several amino acids were found to vary independently. The concentrations of free histidine and valine, and the density of competent larvae in the water, were found to explain 90% of the quantitative temporal variation in larval settlement ($P < .0001$). The mechanism underlying this predictive association is under investigation.

Chemical factors controlling settlement and metamorphosis were found to interact in a complex manner with hydrodynamic effects and other effects of surface roughness and surface relief, in quantitative settlement experiments conducted in the ocean. These interactions include a potentially useful and seemingly paradoxical effect of water flow, surface relief and surface roughness on larval settlement. Quantitative resolution of the interactions between hydrodynamic effects on larval transport and retention, and larval thigmotactic and chemosensory responses, explains this apparent paradox.

PUBLICATIONS:

1. Jensen, R.A. and D.E. Morse. 1988. The bioadhesive of *Phragmatopoma californica* tubes: a silk-like cement containing L-DOPA. *J. Comp. Physiol. B.* 158:317-324.

2. Morse, D.E. and A.N.C. Morse. 1988. Learning from larvae. *Oceanus* 31(3):37-43 + cover.

3. Morse, D.E. 1988. Trigger and amplifier pathways: Sensory receptors, transducers and molecular mechanisms controlling larval settlement, adhesion and metamorphosis in response to environmental chemical signals. In: *Marine Biodeterioration*, M.-F. Thompson, R. Sarojini & R. Nagabhushanam, eds., Amer. Inst. Biol. Sci., Washington, pp. 453-462.

4. Morse, A.N.C. 1988. The role of algal metabolites in the recruitment process. In: *Marine Biodeterioration*, M.-F. Thompson, R. Sarojini & R. Nagabhushanam, eds., Amer. Inst. Biol. Sci., Washington, pp. 463-473.

5. Morse, D.E. 1990. Recent progress in larval settlement and metamorphosis: Closing the gaps between molecular biology and ecology. *Bull. Mar. Sci.* 46:465-483.

6. Jensen, R.A. and D.E. Morse. 1990. Chemically induced metamorphosis of polychaete larvae in both the laboratory and ocean environment. *J. Chem. Ecol.* 16:911-930.

7. Jensen, R.A., D.E. Morse, R.L. Petty and N. Hooker. 1990. Artificial induction of larval metamorphosis by free fatty acids. *J. Exp. Mar. Biol. Ecol.* 67:55-71.

8. Morse, A.N.C. 1991. How do planktonic larvae know where to settle? *Amer. Scientist* 79:154-167.

9. Jensen, R.A. 1991. Marine bioadhesive: Role for chemosensory recognition in marine invertebrates. *Biofouling* (in press).

10. Morse, D.E. 1991. Morphogens, signal molecules and other non-toxic bioactive substances that play a role in structuring interactions and distributions in the marine environment. In: *Marine Bioactive Substances*, ed. by M.-F. Thompson, R. Sarojini and R. Nagabhushanam, Oxford Press, Delhi (in press).

11. Jensen, R.A. 1991. Bioadhesion and morphogenesis in a marine polychaete. In: *Marine Bioactive Substances*, ed. by M.-F. Thompson, R. Sarojini and R. Nagabhushanam, Oxford Press, Delhi (in press).

12. Ilan, M., R.A. Jensen and D.E. Morse. 1991. Modulation of calcium, calcium channels, and protein kinase C, and its effect on a polychaete larval metamorphosis. For: *Exper. Zool.* (submitted).

13. Waite, H., R.A. Jensen and D.E. Morse. DOPA-containing peptide sequences from the adhesive protein of a marine polychaete. For: *J. Biol. Chem.* (in prep.).

STATISTICAL SUMMARY:

Papers in Refereed Journals	9
Technical Reports	3
Books and Chapters	4
Patents Filed	0
Patents Granted	0
Presentations Invited	14
Presentations Contributed	5

	Total	Female	Minority	Non-US citizen
Number of graduate students:	2	1	0	0
Number of postdoctoral fellows:	3	1	0	1

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